Troubleshooting Guide

Problem	Likely Cause	Suggestions
Low DNA yield	Incomplete resuspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Frozen Tissue samples not mixed properly after thawing.	Thaw the frozen Tissue at room temperature an d gently mix the Tissue by inverting.
	Loss the Mag-Bind® particle during operation	Carefully avoid remove the Mag-Bind® particles during aspiration
	DNA remains bound to Mag-Bind®Particles	Increase elution volume and incubate at 65oC for 5 min elution. Pipet up and down for 50-100 times.
Problem in downstream applications	Ethanol carry-over	Dry the Mag-Bind particle before elution.

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E-Z 96® MagSi DNA Tissue DNA Kit for Buccal Swabs

The following protocol is designed for isolating DNA from buccal swabs with magnetic beads.

1. **Place the swab into each well of 96 well plate(no provided).**

2. **Prepare a Proteinase K/Buffer TL working solution by mixing 20 µl Proteinase K with 250µl Buffer TL for each sample.** For each 96 sets of sample, prepare the Protease/TL stock working solution by mix 2.0mL protease with 25mL Buffer TL. Pipet 250 µl protease/TL working solution into each well. Seal the plate properly.

3. **Incubate at 56°C for 1 hours.** Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

4. Spin at 3,000-5,000 x g for 1 minutes at room temperature.

5. Transfer 150 µl of the Sample into each well of Plate (provided).

6. **Add 150µl Buffer MSL, 20 µl MagSi® DNA Paritcles and 220 µl absolute ethanol to the lysate.** Mix the sample by pipetting up and down 20-30 times to mix well or shaking to mix well.
   Note: Buffer MSL, MagSi DNA Particles and absolute ethanol can be premixed. The mixture can be stored at room temperature for 3 hours.

7. **Place the Plate on a magnetic separation device (MSD-01) to magnetize the MagSi DNA particles for 3 min.** The liquid should be cleared after all the magnetic beads are pelleted on the corner of the each well adjecent to the magnet.

8. **Aspirate and discard the cleared supernatant.**

9. Remove the Plate containing the MagSi DNA particles from the magnetic separation device. Add 500µl Buffer MP **diluted with ethanol** into each well.
   Note: Buffer MP must be diluted with absolute ethanol (96-100%) before use in this protocol.

10. Resuspend MagSi DNA particles pellet by pipetting up and down 20 times or shaking to mix well.

Kit Contents

Product Number	M6229-00	M6229-01	M6229-02
Purification Times	1 x 96 Preps	4 x 96 Preps	20 x 96 Preps
500 µl Micro Plate	1	4	20
1.2ml Process Plate	1	4	20
8-strip caps	10 x 8	35 x 8	170 x 8
MagSi DNA Particles	2.2 ml	9 ml	45ml
Buffer TL	20 ml	90 ml	2 x 180 ml
Buffer MSL	20 ml	90 ml	2 x 180 ml
Buffer MP	30 ml	100 ml	8 x 100 ml
Proteinase K	2 ml	8 ml	32 ml
RNase A	550 µl	2.1 ml	10 ml
Elution Buffer	100 ml	2 x 200 ml	8 x 250 ml
Instruction Booklet	1	1	1

E-Z 96® MagSi DNA Tissue DNA Kit for Cultured cells:

This protocol is designed for the rapid isolation of up to 10 ug genomic DNA from up to 1 x 10^6 cultured cells.

1. **Prepare a RNase/PBS working solution by mixing 5 µl RNase A with 100µl PBS for each sample.** For each 96 sets of sample, prepare the Rnase/PBS stock working solution by mix 0.5 mL RNase with 10 mL PBS.

2. **Prepare the cell suspension**
   2a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation, wash the cells with PBS and resuspend cells with 100µl cold (4°C) PBS/RNase. Proceed with step 2 of this protocol.

   2b. For cells grown in suspension, pellet 1 x 10^6 cells by spinning at 1200 x g. Discard the supernatant and wash the cells once with PBS. Resuspend cells with 100µl cold (4°C) PBS/RNase.

   2c. For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or scrape with rubber policemen. Wash cells twice, and resuspend the cells with 100µl cold (4°C) PBS/RNase.

3. **Transfer the suspended cells into each well of Process plate.** Make a chart to record the position of each sample.

4. Add 20 µl Proteinase K into each well of process plate. mix well by shaking. incubate at room temperature for 10 minutes.

5. **Add 100 µl Buffer MSL into each well of Process plate**. Seal the plate properly using the caps supplied. **Incubate at 65°C for 15 min.**

6. Add 20 µl MagSi DNA Particles and 160µl absolute ethanol into each well of process plate. Mix the sample by pipetting up and down 20-30 times to mix well or shaking to mix well.

7. Following steps 8-24 at pate 6-7.

E-Z 96® Mag-Bind Tissue DNA Protocol for Tissues

Materials to be provided by user:

- Laboratory centrifuge capable of 3,000-5,000 x g equipped with swinging-bucket rotor.
- Adapter for deep-well microplate
- Waterbath equilibrated to 56°C and 65°C
- Equilibrated sterile dH_2O water or Elution Buffer at 65°C
- Absolute (96%-100%) ethanol
- Multichannel pipet with tips
- E-Z 96 Magnetic Separation Strand **(Cat# MSD-01)**

OPTIONAL: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue to a clean Racked Microtubes. Add 130 µl Buffer TL/**Proteinase** K and proceed to step 3 below.

1. **Mince 5-10 mg tissues and place into each well of 1.2ml process plate.** Make a chart to record the position of each sample.

2. **Prepare an Proteinase K/Buffer TL working solution by mixing 20 µl Proteinase K with 130 ul Buffer TL for each sample.** For each 96 sets of samples, prepare the Protease/TL stock working solution by mix 2.0 mL protease with 13 mL Buffer TL. Pipet 130 µl protease/TL working solution into each well. Seal the plate properly using the caps supplied.

3. **Mix the samples by inverting the plate 3-5 times.** Briefly spin the plate at 2,500-3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution.

4. **Incubate at 56°C overnight or until the samples are completely lysed.** The lysate should be clear and viscous after digestion is complete. Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

5. **Shake or vortex the plate vigorously from side to side.** (Do not shake up and down to avoid leaking around the caps.) Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

Introduction

The E-Z 96® Mag-Bind® Tissue DNA Kit provides a rapid and easy method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 10 mg tissue or up to 1 cm sections of mouse tail can be readily processed in one time. The method can also be used for preparation of genomic DNA from mouse tail snips, blood, buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps such as precipitation with isopropanol or ethanol are eliminated. The Kit allows single or multiple simultaneous processing of samples in under 1 hours. DNA purified using The E-Z 96® Mag-Binds® Tissue DNA Kit is ready for applications such as PCR, Southern blotting and restriction digestion.

Overview

If using the E-Z 96® Mag-Binds® Tissue DNA Kit for the first time, please read this booklet in its entirety to become familiar with the procedures. Tissue samples lysed in a specially formulated buffer. The binding conditions are adjusted so that genomic DNA will selectively bind to the Mag-Binds®particles. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

Most components of the E-Z 96® Mag-Bind® Tissue DNA Kit, except RNase A, Proteinase K are stable for at least 24 months from date of purchase when stored at 22-25ºC. MagSi DNA Particles Solution should be stored at 4°C for long-term use. Store RNase A at -20° C. Proteinase K should be stored at 15-25ºC. During shipment or storage in cool ambient conditions, precipitates may form in Buffer MSL and Buffer TL. Dissolve such deposits by warming the solution at 37ºC and gently shaking.

11. **Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min.** Remove and discard the cleared supernatant.

12. Remove the plate from the magnetic separation device. **Add 500µl of 70% ethanol and pellet by pipetting up and down 5 times or shaking to mix well.**

13. Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min. Remove and discard the cleared supernatant.

14. Aspirate and discard the cleared supernatant.

15. Remove the plate from the magnetic separation device. **Add 500µl of 70% ethanol into the plate and pellet by pipetting up and down 5 times or shaking to mix well.**

16. Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min. Remove and discard the cleared supernatant.

17. Remove and discard the cleared supernatant.

18. Do not remove the plate from the magnetic separation device. Add 500µl Elution Buffer into the plate. incubate for 30 seconds. Remove and discard the cleared supernatant.

19. Remove the Plate from magnetic separation device. **Add 50-100µl Elution Buffer preheated at 70°C to elute DNA from the magnetic particles.**

20. **Resuspend MagSi DNA particles by pipetting up and down 20-30 times or shaking to mix.** Incubate 5 minutes at 70oC or 15 minutes at room temperature.

21. **Place the plate onto magnetic separation device to magnetize the Mag-Bead® particles.** Wait 7–10 minutes or until magnetic beads are cleared from the solution.

22. **Transfer the cleared supernatant containing purified DNA to a new 500µl microplate (suplied).**

23. Seal the plate with sealing film store at -20°C.

Before Starting

- Please read this booklet thoroughly to become familiar with the E-Z 96® MagSi DNA Tissue DNA Kit procedures.
- Equilibrate Elution Buffer (or sterile dH_2O water or 10 mM Tris pH 9.0) at 65°C.
- Dilute Buffer MP with absolute ethanol as follows and **store at room temperature**.

M6229-00	Add 75 ml absolute (96%-100%) ethanol
M6229-01	Add 150 ml absolute (96%-100%) ethanol
M6229-02	Add 150 ml absolute (96%-100%) ethanol to each bottle

E-Z 96 MagSi DNA Tissue DNA Kit for Mouse Tail Snips

1. **Snip one piece of mouse tail 0.1 - 0.2 cm in length, place into a new Process plate.** If necessary cauterize the wound to stop bleeding.

   Note: Mice should not be older that 6 weeks, since lysis will be more difficult resulting in suboptimal DNA yields. If possible, obtain tail biopsy at 2-4 weeks and freeze samples at - 70°C until DNA is extracted.

2. **Prepare a Proteinase K/Buffer TL working solution by mixing 20 µl Proteinase K with 180µl Buffer TL for each sample.** For each 96 sets of sample, prepare the Protease/TL stock working solution by mix 2.0mL protease with 18mL Buffer TL. Pipet 200 µl protease/TL working solution into each well. Seal the plate properly using the caps supplied.

3. **Mix the samples by inverting the plate.** Briefly spin the plate at 2,500 - 3,000 x g to collect any residue solution from the caps. It is very important that samples are completely submerged in the solution. If the protease/TL solution does not completely cover the sample, increase the sample volume to 200µl. (Additional reagent can be purchased separately).

4. **Incubate at 56°C over night or until the samples are completely lysed.** The lysate should be clear and viscous after digestion is complete. Mix occasionally during the incubation by rotating the plate gently. Make sure the samples are completely lysed.

5. **Shake or vortex the plate vigorously from side to side.** (Do not shake up and down to avoid leaking around the caps.) Hold the caps to ensure the plate is sealed properly. Ensure the lysate is completely homogenous after shaking. If a gelatinous mass is visible, further digestion is required.

6. **Optional: For isolation of RNA-Free genomic DNA, spin briefly to collect any drops and add 5µl RNase A solution (20mg/mL) to each sample** and incubate 10-20 minutes to remove the RNA.

7. **Spin at 3,000-5,000 x g for 10 minutes at room temperature to remove undigested particles.**

8. **Transfer 150µl of the supernatant into each well of Process Plate.**

9. Following step 7-24 on page 6-7.

6. **Optional:** Certain tissues such as liver have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. For isolation of RNA-Free genomic DNA, spin briefly to collect any drops and add 5µl RNase A solution (20mg/mL) to each sample and incubate 10-20 minutes to remove the RNA.

7. **Add 150 ul Buffer MSL, 20 µl MagSi® DNA Paritcles and 220 µl absolute ethanol to the lysate.** Mix the sample by pipetting up and down 20-30 times to mix well or shaking to mix well.
   Note: Buffer MSL, MagSi DNA Particles and absolute ethanol can be premixed. The mixture can be stored at room temperature for 3 hours.

8. **Place the Plate on a magnetic separation device (MSD-01) to magnetize the MagSi DNA particles for 3 min.** The liquid should be cleared after all the magnetic beads are pelleted on the corner of the each well adjecent to the magnet.

9. **Aspirate and discard the cleared supernatant**.

10. Remove the Plate containing the MagSi DNA particles from the magnetic separation device. Add 500µl Buffer MP **diluted with ethanol** into each well.
    Note: Buffer MP must be diluted with absolute ethanol (96-100%) before use in this protocol.

11. Resuspend MagSi DNA particles pellet by pipetting up and down 20 times or shaking to mix well.

12. **Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min.** Remove and discard the cleared supernatant.

13. Remove the plate from the magnetic separation device. **Add 500µl of 70% ethanol and pellet by pipetting up and down 5 times or shaking to mix well.**

14. Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min. Remove and discard the cleared supernatant.

15. Aspirate and discard the cleared supernatant.

16. Remove the plate from the magnetic separation device. **Add 500µl of 70% ethanol into the plate and pellet by pipetting up and down 5 times or shaking to mix well.**

17. Place the plate onto a magnetic separation device to magnetize the MagSi DNA particles for 3 min. Remove and discard the cleared supernatant.

18. Remove and discard the cleared supernatant.

19. Do not remove the plate from the magnetic separation device. Add 500µl Elution Buffer into the plate. incubate for 30 seconds. Remove and discard the cleared supernatant.

20. Remove the Plate from magnetic separation device. **Add 50-100µl Elution Buffer preheated at 70°C to elute DNA from the magnetic particles.**

21. **Resuspend MagSi DNA particles by pipetting up and down 20-30 times or shaking to mix well.** Incubate 5 minutes at 70oC or 15 minutes at room temperature.

22. **Place the plate onto magnetic separation device to magnetize the Mag-Bead® particles.** Wait 7–10 minutes or until magnetic beads are cleared from the solution.

23. **Transfer the cleared supernatant containing purified DNA to a new 500µl microplate (suplied).**

24. Seal the plate with sealing film store at -20°C.